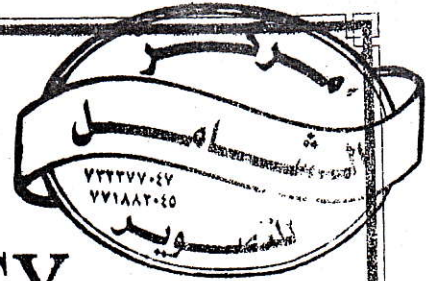


CLINICAL
MICROBIOLOGY
NON SPORE FORMING
GRAM - POSITIVE RODS.
ACTINOMYCETES RELATED
- ORGANISMS.



→ Genus: *Corynebacterium* ←



FIG. 91. "Bull-neck" diphtheria—solid and relatively painless oedema of the neck.

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23.2.15.

Diphtheria → Greek
Hide membrane

ACTINOMYCETES RELATED ORGANISMS

- Genus: **Corynebacterium**.

SPECIES:

1. *Corynebacterium diphtheriae*. Causes Diphtheria in man only.

2. *Corynebacterium pseudotuberculosis*. (Preis - Nocard - Bacillus). Ulcerative Lymphangitis, abscesses, other chronic - purulent infections in sheep, goats, horses, and man.

3. *Corynebacterium xerosis* (non - pathogenic: found in conjunctival - sac in man + skin and mucous membranes).

4. *Corynebacterium renale*: non - pathogenic for man.

Causes cystitis and pyelonephritis in cattle.

5. *Corynebacterium pseudodiphthericum*:

- Not pathogenic for man.

- Found in nasopharyngeal mucosa of man.

6. *Corynebacterium kutscheri*: pathogenic for rats and mice.

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inflammation of kidney and renal pelvis usually due to ascending infection from urinary bladder.

7. *Corynebacterium equi* : causes bronchopneumonia in foals + abortion in cows.

8. *Corynebacterium bovis* : can cause bovine – mastitis.

9. *Corynebacterium paurometabolum* : isolated from ovaries of bed- bug!

10. *Corynebacterium pyogenes*. Causes pyogenic – infections in cattle, sheep and pigs.

11. PLANT – PATHOGENIC CORYNEBACTERIA.

a. *Corynebacterium fascians*.

b. *Corynebacterium rathayi*.

c. *Corynebacterium agropyri*.

d. *Corynebacterium tritici*.

e. *Corynebacterium iranikum*.

12. 18 – Species of other non – pathogenic corynebacteria.

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NON SPORE FORMING GRAM – POSITIVE RODS

Two Important Pathogens :

1. Corynebacterium diphtheriae.

And 2. Listeria monocytogenes.

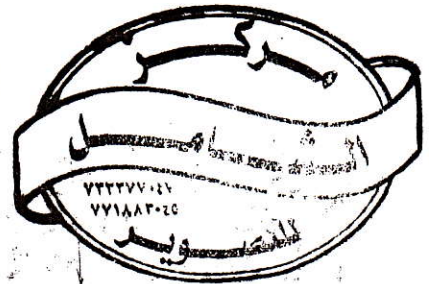
Corynebacterium Diphtheriae :

Disease : C. diphtheriae causes Diphtheria; But Diphtheroids are implicated in opportunistic infections. (Diphtheria From The Greek – Word For Leather).

Important – Properties : Corynebacteria are Gram – Positive rods that appear club – shaped (wider at one end) and are arranged in Palisades (side by side arrangement) or in V-Y-L or Z- shaped formations (chines – letters). The rods have a beaded appearance. The beads consist of granules of highly polymerized polyphosphate , a storage mechanism for high energy phosphate bonds. The granules stain **METACHROMATICALLY** ; i.e. a dye that stains the rest of the cell blue while stains the granules red, (**NOT ALBERT'S STAIN**).

TRANSMISSION :-

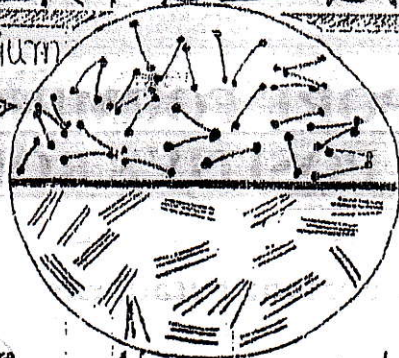
1. Pure human infection .
2. Toxigenic and Non – Toxigenic strains reside in the U.R. tract.
3. Airoborne droplet mode of transmission .
4. Can infect skin in tropical areas in persons with poor skin hygiene.



The diagnostic feature is the elevated 'wash leather' greyish green membrane on the tonsils

ALBERT'S STAIN

Corynebacterium
diphtheriae →



Non-pathogenic
diphtheroids ←

Culture

- 1/ Growth occurs better in the presence of blood or serum (Loeffler's medium).
- 2/ On the selective medium (Potassium tellurite medium = Hoyle's medium),
- 3 epidemiological types can be differentiated:
 - 1/ Type gravis = Colonies are large, grey-black, matt, daisy-head.
 - 2/ Type intermedius = Colonies are very small, flat and black.
 - 3/ Type mitis = Colonies are large, grey black, with a glossy surface, and regular outline.

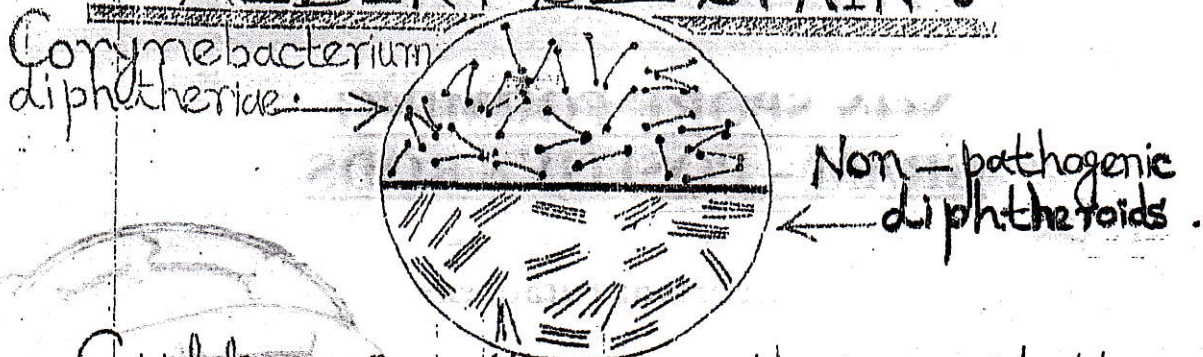
BIOCHEMICAL PROPERTIES

- 1/ Glucose = Acid production.
- 2/ Saccharose = Negative reaction.
- 3/ Starch = Positive reaction for type gravis, and negative reaction for type mitis.
- 4/ Urea-Test = Negative reaction.

N.B. To all the above-mentioned sugars and media add a few drops →

The diagnostic feature is the elevated 'wash leather' greyish green membrane on the tonsils

ALBERT'S - STAIN



Culture :- 1/ Growth occurs better in the presence of blood or serum (Loeffler's medium). 2/ On the selective medium (Potassium tellurite medium = Hoyle's medium), 3 epidemiological types can be differentiated:-
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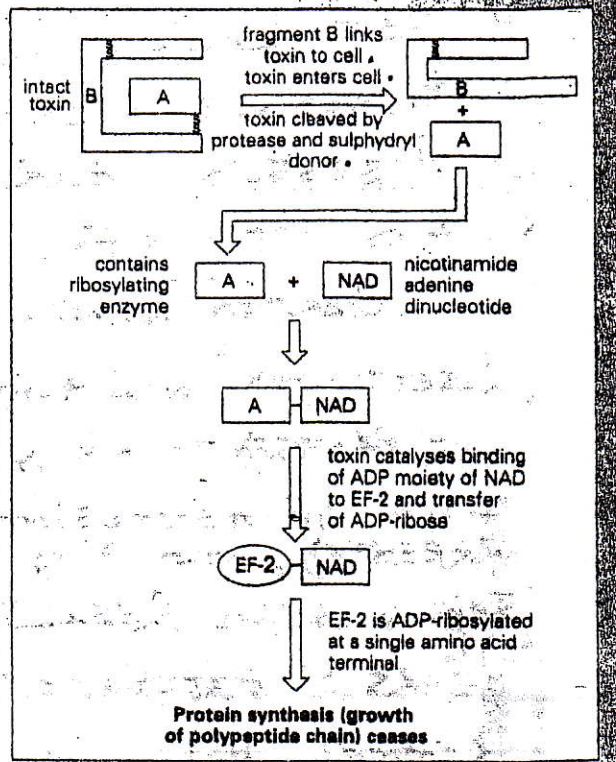
OF HORSE - SERUM (AS AN ENRICHED) - FACTOR). **PATHOGENESIS:-**

Diphtheria toxin :

The genes encoding toxin production are carried by a temperate bacteriophage which, during the lysogenic phase, is integrated into the bacterial chromosome. The toxin is synthesized as a single polypeptide (mol. wt 62 000; 535 amino acids), consisting of fragment B (binding) at the carboxyterminal end, which attaches the toxin to the host cells (or to any eucaryotic cell), and fragment A (active) at the aminoterminal end, which is the toxic fragment. Toxic fragment A is only formed (by protease cleavage and reduction of disulphide bonds) after uptake of the toxin into the cell. Fragment A inactivates elongation factor-2 (EF-2) by ADP-ribosylation, and thereby inhibits protein synthesis (Fig. 20.16). Prokaryotic and mitochondrial protein synthesis is not affected because a different elongation factor is involved. A single bacterium can produce 5000 toxin molecules per hour and the toxic fragment is so stable within the cell that a single molecule can kill a cell. For unknown reasons myocardial and peripheral nerve cells are particularly susceptible.

*myocarditis
peripheral neuropathy*

Fig 20.18 Diphtheria toxin - mechanism of action.



HOW BACTERIAL PATHOGENS DAMAGE HOST CELLS

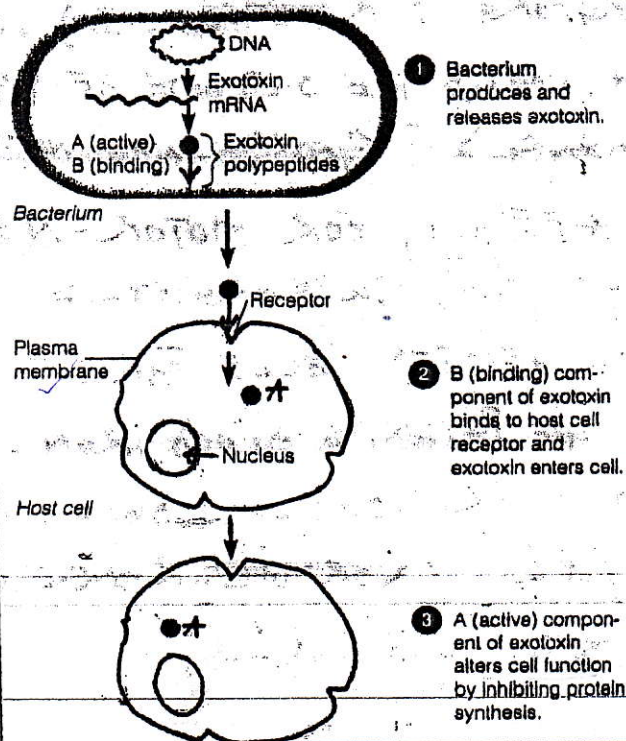


Figure 15.4 The action of an exotoxin. A proposed model for the mechanism of action of diphtheria toxin.

Diphtheria toxin consists of two different polypeptides: B (binding) and A (active); B is required to make A active.

Elongation factor-2

Clinical picture of scarlet fever?

- 6 -

OTHER PROPERTIES OF DIPHTHERIA

- TOXIN :-

- 1/. HEAT - ^{changeable} LABILE - EXOTOXIN.
- 2/. POSSESSES NECROTIC - EFFECTS. *Mysocarditis*
- 3/. POSSESSES NEUROTIC - EFFECTS. *Peripheral neuropathy*
- 4/. HAS AFFINITY FOR CARDIAC - MUSCLES :-

GERMAN GENERAL ERWIN - ROMMEL, the "desert-fox", WAS ALWAYS SEEN WITH A HANDKERCHIEF PRESSED TO HIS NOSE. ROMMEL ARRANGED A PLOT TO ASSASSINATE HITLER. ROMMEL WAS ARRESTED, AND DIED OF A HEART - ATTACK. THIS WAS THE RESULT OF A WEAKENED HEART BY YEARS OF EXPOSURE TO DIPHTHERIA - TOXIN - ROMMEL HAD NASAL - DIPHTHERIA.

- THEREFORE IF ROMMEL HAD NEVER CONTRACTED DIPHTHERIA, WOULD THE OUTCOME OF WORLD - WAR - II HAVE CHANGED? GEORGE - WASHINGTON ALSO DIED FR. - DIPHTHERIA
- 5/. HAS AFFINITY FOR MOTOR - NERVE - ENDING, KIDNEYS AND SUPRARENALS.
 - 6/. PHAGE - INFECTED CELLS FAILS TO PRODUCE TOXIN UNLESS THE PATIENT'S BLOOD IRON - CONCENTRATION DROPS TO A CRITICALLY LOW - LEVEL -, BELOW $30 \mu\text{g/dL}$. (NORMAL - RANGE OF SERUM - IRON = $250 - 350 \mu\text{g/dL}$).

- 6 -

*Handkerchief
a piece of cloth that you use to
dry your nose.*

- 7 -
- 7/. CURING THE BACTERIUM OF ITS PHAGE - INFECTION, ELIMINATES ITS ABILITY TO PRODUCE TOXIN ; (IN VITRO BY ANTIVIRAL - DRUGS).

PORT OF ENTRY:-

- 1/. UPPER - RESPIRATORY - TRACT : THE MOST COMMON MODE OF TRANSMISSION - AIR - DROPLETS - MODE OF TRANSMISSION.

RARE MODES OF TRANSMISSIONS:-

- 1/. THROUGH WOUNDS AND SKIN.
- 2/. THROUGH THE EARS.
- 3/. THROUGH THE VAGINA.
- 4/. THROUGH THE NOSE.
- 5/. THROUGH THE EYES.



I. TARGET - ORGANS:

1/ TONSILS 2/ PHARYNX AND 3/ LARYNX.

II. TYPES OF LESIONS:

1/ ARREST OF PROTEIN - SYNTHESIS.

2/ NECROTIC AND NEUROTOXIC - EFFECTS ON TISSUES.

RESPIRATORY - DIPHTHERIA:

Corynebacterium diphtheriae is NOT-INVASIVE; IT DOES NOT INVADE DEEP TISSUES AND NEVER ENTERS THE BLOOD-STREAM. DIPHTHERIA DOES NOT OCCUR NATURALLY IN ANIMALS.

1/ IN THE TARGET-ORGANS *Corynebacterium diphtheriae* bacilli multiply AND SECRETE EXOTOXIN. THIS TOXIN PRODUCES AN AREA OF NECROSIS. THE RESULTING FIBRINOUS-EXUDATE TOGETHER WITH EPITHELIAL-CELLS, WBC, RBC AND BACTERIA FORM THICK, GRAY, ADHERENT-MEMBRANE (PSEUDOMEMBRANE) OVER THE TONSILS AND THROAT. (STRONGLY ADHERENT TO SQUAMOUS EPITHELIUM BUT LOOSELY ATTACHED TO CILIATED-EPITHELIUM).

2/ EXTENSION OF THE MEMBRANE INTO THE LARYNX

AND TRACHEA, CAUSING AIRWAY OBSTRUCTION. ^{URGENT-LIFE-SAVING} ~~tracheostomy~~

3/ MYOCARDITIS ACCOMPANIED BY ARRHYTHMIAS AND CIRCULATORY-COLLAPSE; AND

4/ RECURRENT-LARYNGEAL-NERVE-PALSY.

CUTANEOUS - DIPHTHERIA CAUSES ULCERATING SKIN LESIONS BUT DOES NOT CAUSE SYSTEMIC-SYMPTOMS.

MOST DIPHTHERIA-LESIONS HAVE A CHARACTERISTIC OFFENSIVE PUNGENT ODOR ⇒ "FOETOR".

IN SEVERE CASES OF RESPIRATORY-DIPHTHERIA, RENAL-FAILURE AND SUPRARENAL-FAILURE ⇒ ACUTE SUPRARENAL-INSUFFICIENCY (WATERHOUSE-FRIEDRICHSEN-SYNDROME) OCCUR WITH FATAL-CONSEQUENCES.

NON - RESPIRATORY - DIPHTHERIA :

1/ WOUND OR SKIN - DIPHTHERIA: OCCURS CHIEFLY IN THE TROPICS. A MEMBRANE IS FORMED ON AN INFECTED - WOUND THAT FAILS TO HEAL AND HAS OFFENSIVE - SMELL DISCHARGE AND CHRONIC - COURSE OF THE DISEASE.

2/ NOSE: EPISODES OF NASAL - BLEEDINGS.

3/ EYES: REDNESS WITH CONJUNCTIVAL - DISCHARGE.

4/ EARS: CHRONIC INFECTION OF THE EARS.

5/ VAGINA: CHRONIC VAGINAL - DISCHARGE WITH OFFENSIVE - SMELL.

CLINICAL - FINDINGS :

DIPHTHERIA IS AN ACUTE - INFECTION WITH A MORTALITY RATE BETWEEN 10% TO 30% AND MUCH HIGHER IN OLDER - PEOPLE OR WHEN TREATMENT HAS BEEN DELAYED.

BOTH TOXIGENIC AND NONTOXIGENIC - STRAINS RESIDE IN THE UPPER RESPIRATORY - TRACT AND ARE TRANSMITTED BY AIRBORNE - DROPLETS.

INCUBATION - PERIOD IS 3 TO 4 DAYS.

PATHOGENECITY AND CLINICAL - PICTURE, CHIEFLY DEPEND ON TOXIN - ACTION OF TOXIGENIC - STRAINS OF *Corynebacterium diphtheriae*.

Diphtheria inflammation begins with sore throat and fever. Dyspnea develops and extension of the membrane into the larynx and trachea will cause airway obstruction needing an URGENT - LIFE - SAVING - TRACHEOTOMY.

In severe, untreated cases, myocarditis, arrhythmias and circulatory - collapse and death occur.

TREATMENT:

1/. Start Diphtheria - Antitoxin (After-Test - Dose - A.T.) - Because the antitoxin is HORSE - ORIGIN, which can lead to FATAL - ANAPHYLACTIC - SHOCK and DEATH). This is the treatment of CHOICE.

2/. Penicillin (A.T.I) OR ERYTHROMYCIN: ANTIBIOTICS INHIBIT GROWTH OF *Corynebacterium diphtheriae*. REDUCE TOXIN - PRODUCTION, AND DECREASE THE INCIDENCE OF CHRONIC - CARRIERS.

3/. TREATMENT OF COMPLICATIONS.



NON-RESPIRATORY DIPHTHERIA

1. Diphtheria of Wounds.
2. Diphtheria of skin-lesions.
3. The organism may grow in the eyes.
4. Diphtheria of genital tract. *Vagina*

TRANSMISSION:

The organism enters our bodies through the respiratory tract and is spread by respiratory secretions from ~~patients~~ **healthy-carriers** with active disease or

LABORATORY-DIAGNOSIS.

Diphtheria must be considered in every case of exudative tonsillitis.

SPECIMENS: — TAKEN BY AN E.N.T. SPECIALIST.

3 — Throat — Swabs from under the pseudomembrane must be taken. One swab for Albert's stain and Gram-stain.

ONE FOR

- AND ONE SWAB FOR CULTURE INTO POTASSIUM-TELLURITE — (HOYLE'S — MEIUM) — MEIUM
AND LOEFFLER'S — MEIUM.

STAPH. STREPT.
CANDIDA
FUSIFORMIS
BACTERIA

- 12 -

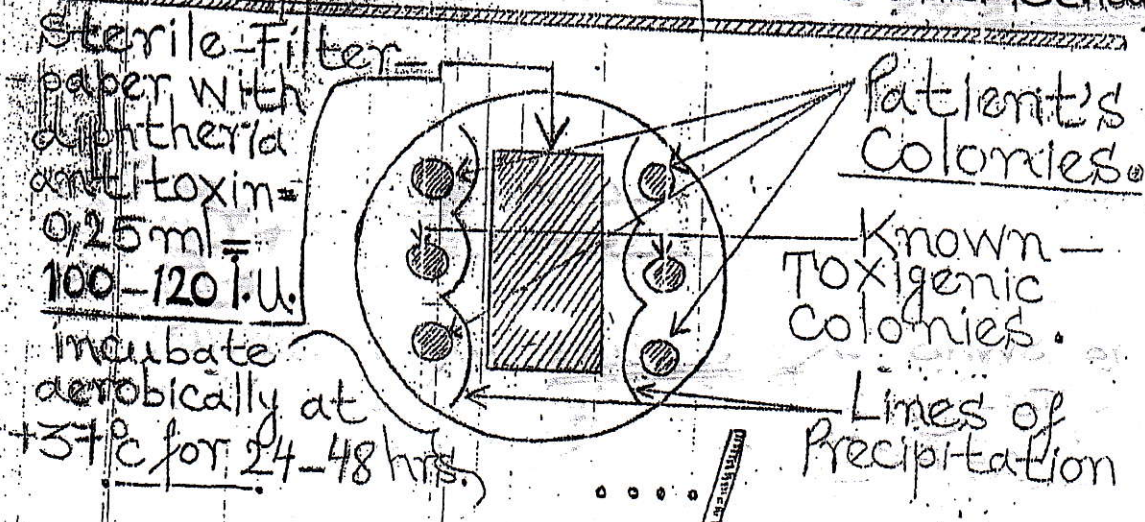
IF YOU FIND MICROSCOPICALLY ANY BACILLI RESEMBLING CORYNEBACTERIA, PHONE THE WARD AND THE DOCTOR IN-CHARGE VERY URGENTLY.

The second swab should be immediately inoculated on to blood agar, Loeffler's medium and Joyle's medium. Incubate aerobically at $+37^{\circ}\text{C}$ for 24 hours. Examine after 6-12 hours, if negative, re-examine after 24 hours.

Do Albert's stain for the suspected colonies. Urgently phone this second result to the Ward.

TOXIGENICITY TEST

(ELEK'S METHOD).
(Gel-Diffusion-Precipitation-Method).



- 12 -



The medium used in Elek's method is 20% horse - serum clear nutrient agar. (**EXTREMELY CLEAR TRANSPARENT AGAR**).

SCHICK TEST :

An intradermal test for the demonstration of immunized and unimmunized persons.

0.2 ML. Of 1/50 of the M.L.D of diphtheria toxin is injected into the skin of the left forearm (test) and heat inactivated toxin into the right (control). Results are read at 48 hrs and again at 1 week.

- + Ve = Red - area = Not - immune.
- Ve = No - reaction = Immune.

∴ This is a toxin - antitoxin neutralization reaction in vivo.

Epidemiology, prevention and control:

1. Active immunization is done with D.P.T. at the age of 2, 4, and 6 months. Booster doses are done at the ages 1 and 6 years of age.

Active immunization with D.P.T. vaccine proved so highly successful that it reduces the incidence and mortality of Diphtheria to zero.

2. Sanitary Epidemiological Investigatory survey to detect carriers.
3. Isolation and treatment of cases.

Note:

Home work: Albert's stain

mention *Bacillus* forgotten in yener
 1 - *Leptospira* 1 ch to new (refuge)
 2 - *Sigella monocyto genes*
 3 - *Acanthamoeba*

14 -

14 -

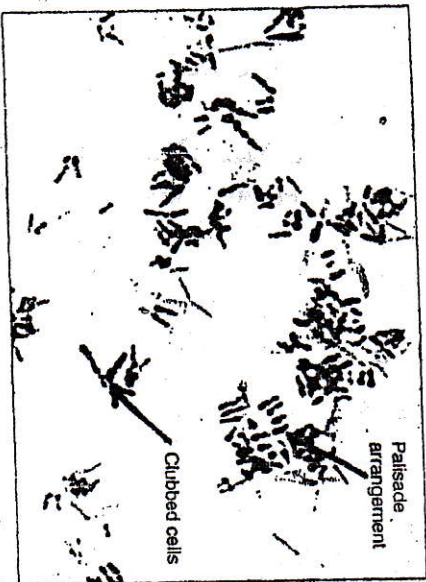


Figure 24.5 *Corynebacterium diphtheriae*, the cause of diphtheria. This Gram stain shows the club-shaped morphology; the dividing cells are often observed to fold together to form V- and Y-shaped figures. Also, notice the side-by-side palisade arrangement.

A lysogenic phage in *C. diphtheriae* carries the gene for diphtheria toxin.

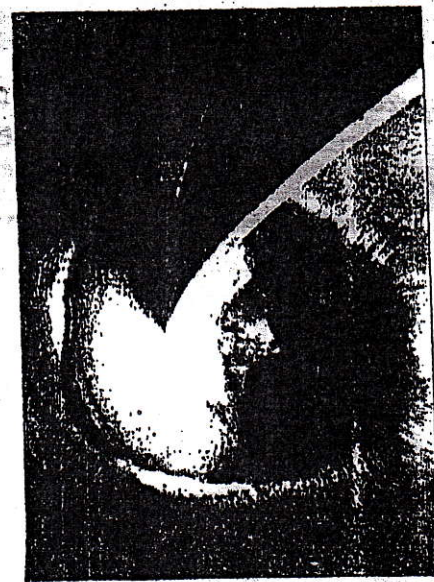


Figure 24.6 A diphtheria membrane. What is cutaneous diphtheria?

Acetabularia and *Naegleria fowleri* rarely
found in human not registered.

LISTERIA MONOCYTOGENES

Specimen
L. monocytogenes

Diseases *L. monocytogenes* causes meningitis and sepsis in newborns and immunosuppressed adults. It also causes outbreaks of febrile gastroenteritis.

Important Properties *L. monocytogenes* is a small gram-positive rod arranged in V- or L-shaped formations similar to corynebacteria. The organism exhibits an unusual **tumbling** movement that distinguishes it from the corynebacteria, which are nonmotile. Colonies on a blood agar plate produce a narrow zone of beta-hemolysis that resembles the hemolysis of some streptococci.

Pathogenesis *Listeria* infections occur primarily in two clinical settings: (1) in the fetus or newborn as a result of transmission across the placenta or during delivery; and (2) in immunosuppressed adults, especially renal transplant patients. The organism is distributed worldwide in animals, plants, and soil. From these reservoirs, it is transmitted to humans by contact with animals or their feces, by unpasteurized milk, and by contaminated vegetables. In the United States, listeriosis is primarily a food-borne disease associated with eating unpasteurized cheese.

The pathogenesis of *Listeria* is dependent upon the organism's ability to invade mononuclear phagocytic cells. Because *Listeria* preferentially grows intracellularly, cell-mediated immunity is a more important host defense than humoral immunity. Suppression of cell-mediated immunity predisposes to *Listeria* infections. *L. monocytogenes* can move from cell to cell by means of "actin rockets," a filament of actin that contracts and propels the bacteria through the membrane of one human cell and into another. **Listeriolysin O** is an important virulence factor. It is a cytolysin/hemolysin similar to streptolysin O that acts by degrading cell membranes.

Clinical Findings Infection during pregnancy can cause abortion, premature delivery, or sepsis during the peripartum period. Newborns infected at the time of delivery can have acute meningitis 1-4 weeks later. The infected mother either is asymptomatic or has an influenzalike illness. *L. monocytogenes* infections in immunocompromised adults can be either sepsis or meningitis.

Gastroenteritis caused by *L. monocytogenes* is characterized by watery diarrhea, fever, headache, myalgias, and abdominal cramps but little vomiting. Outbreaks are usually caused by contaminated dairy products, but undercooked meats such as chicken and hot dogs have also been involved.

Laboratory Diagnosis Laboratory diagnosis is made primarily by Gram stain and culture. The appearance of gram-positive rods resembling diphtheroids and the formation of small, gray



Postnatal vector
of Milk

Cause
Postnatal
sepsis

Flagyl
Anti-anaerobic
Bacteria

Forgotten
Bacteria

Leptospirosis

Leptospira interrogans

Q fever

END

Q fever
Jandrić

colonies with a narrow zone of beta-hemolysis on a blood agar plate suggest the presence of *Listeria*. The isolation of *Listeria* is confirmed by the presence of motile organisms, which differentiate them from the nonmotile corynebacteria. Identification of the organism as *Listeria monocytogenes* is made by sugar fermentation tests.

Treatment Treatment of invasive disease consists of ampicillin with or without gentamicin. Trimethoprim-sulfamethoxazole is also effective. Resistant strains are rare. *Listeria* gastroenteritis typically does not require treatment.

Prevention Prevention is difficult because there is no immunization. Limiting the exposure of immunosuppressed patients to potential sources such as infected animals and their products and contaminated vegetables is recommended.

REVIEW QUESTIONS

1. How is the capsule of *Bacillus anthracis* different from that of most other bacteria?
2. What is the natural habitat of *B. anthracis*, and how does it persist there for long periods?
3. How does *B. anthracis* cause disease?
4. *Bacillus cereus* causes food poisoning. How is it transmitted? What is the pathogenesis?
5. How is tetanus acquired? What is its pathogenesis?
6. What are the three important components of tetanus prevention?
7. How is botulism acquired? What is its pathogenesis? How is it prevented?
8. *Clostridium perfringens* can cause gas gangrene. Under what circumstances does this occur, and what is the role of alpha toxin in the disease? How can the disease be prevented?
9. In an exudate from a patient with gas gangrene, what would you see on Gram stain? How would you culture the organism?
10. What is the pathogenesis of food poisoning caused by *C. perfringens*?
11. What is the pathogenesis of diarrhea caused by *Clostridium difficile*?
12. What is the appearance of *Corynebacterium diphtheriae* on Gram stain?
13. What is the mechanism of action of diphtheria toxin?
14. Only lysogenized strains of *C. diphtheriae* are pathogenic. Why?
15. Why is diphtheria rare in the United States?
16. What is the role of antitoxin in the treatment of diphtheria?
17. In which two population groups do *Listeria* infections primarily occur?
18. How is *Listeria monocytogenes* transmitted, and what is its mode of pathogenesis?